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16. ABSTRACT

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Chapter 6. QUANTITATIVE EXPRESSION OF BIOTRANSFORMATION

Quantitative Expression of Biotransformation Rate

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Methodologies and approaches to the study of microbial transformation of organic compounds in natural waters and sediments are outlined. Tentative evidence is given to demonstrate that use of rate constants is a reasonable approach. Application of second-order kinetics incorporating the effects of organic compound concentration, bacterial concentration, and sorption by suspended sediment is made to natural systems.

It is now generally conceded that hazard assessment requires some understanding of the fate of chemicals in the aquatic ecosystem. This requirement stems from the need to forecast the exposure concentration in order to predict impact. The problems and approaches to hazard assessment were the subject of another meeting at Pellston, Mich., in 1977 (6). At that time, it was pointed out (3, 5) that environmental concentrations of a pollutant could be predicted if the rates of important processes controlling pollutant concentration can be determined. This is easily summarized by equation 1.

$$\text{rate}_{net} = \text{rate}_{in} - \text{rate}_{export} - \text{rate}_{abiotic} - \text{rate}_{biological} \quad (1)$$

Here, the terms represent the net rate of change in pollutant concentrations, rates of change due to input, export and volatilization, abiotic transformation, and biological transformation, respectively. It is not our purpose here to examine this hypothesis and its associated assumptions but rather to discuss the feasibility of adequately describing the final term in equation 1, i.e., the kinetics of microbiological transformation.

DISCUSSION

Microbial kinetics is not a new area of research, having been used for many years in fermentation processes, drug manufacture, and waste treatment (1, 2). In most cases, however, the approaches have relied on the empirical equation first suggested by Monod (10) (equation 2) or modification thereof.

$$\frac{d[B]}{dt}\left(\frac{1}{[B]}\right) = u = \frac{u_m[S]}{K_s + [S]} \quad (2)$$

where $[B]$ is microbial population or activity per unit volume, $[S]$ is substrate concentration, u_m and u are maximum specific and specific growth rates, respectively, and $K_s = [S]$ when $u = \frac{1}{2}u_m$.

Because so much microbial kinetic work has been done using this equation,

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a brief assessment of its suitability for environmental purposes is in order. This equation has usually been modified (equation 3) by addition of a yield coefficient ($Y = d[B]/d[S]$) which simply describes the efficiency with which substrate is converted to biomass because substrate transformation rate is required rather than growth rate.

$$\frac{d[B]}{dt}\left(\frac{d[S]}{d[B]}\right) = -\frac{d[S]}{dt} = \frac{u_m[B][S]}{Y(K_s + [S])} \tag{3}$$

The practical difficulty of basing kinetics on measured values of Y is not obvious except in a system having a single limiting substrate. Under these conditions, Y must approach zero as $[S]$ approaches zero because of maintenance metabolism. In nature, however, the pollutant is not the sole substrate, and so the proper value or values of Y are not readily determined from laboratory studies.

Because the condition of chief concern is one of long-term, low-level input to the environment, the concentration of substrate can be assumed to be much less than K_s. This does not seem too unreasonable since values for K_s typically range from 0.1 to 10 mg/liter, which is much higher than the concentration of most pollutants. Under this circumstance, equation 3 can be approximated by equation 4, which is in the familiar form of second-order kinetics.

$$-\frac{d[S]}{dt} = \frac{u_m}{YK_s}[B][S] = k[B][S] \tag{4}$$

Equivalence of u_m/YK_s and k has been demonstrated experimentally for systems where the substrate was the sole carbon source and second-order kinetics were observed (11, 12).

The rationale for use of a second-order rate expression derived from the Monod equations is thus tentative, but it is certainly well known that microbial rates are a function of population size and substrate concentration at higher concentrations. The possibility of a threshold at low concentrations has been speculated on by Boethling and Alexander (4), but the data are inconclusive. These equations also neglect the problem of acclimation or induction and describe the rate only after such processes have occurred. This does not, however, seem inconsistent with the need to predict rates that might occur in a system receiving input over a long period of time.

Tentative acceptance of a second-order equation as the appropriate phenomenological description leaves a number of questions of a conceptual, and thus experimental, nature. How is $[B]$ to be defined and measured? How is the substrate defined and measured, i.e., CO_2 equivalence, specific compound analysis, chemical oxygen demand, etc.? Assuming that the kinetics are second order and that the rate constant can be measured for a natural system, what is the variability in the rate constant from one ecosystem to another? These questions will now be addressed in reverse order.

Table 1 shows second-order rate constants that were measured (D. F. Paris, W. C. Steen, and G. L. Baughman, Natl. Meet. Am. Chem. Soc., 175th, Anaheim, Calif., March 1978) for a number of different waters. The results were obtained by the river die-away method, except that plate counts were used to determine population size, and substrate concentration was maintained low enough so that the kinetics were pseudo-first order in substrate. Examination

TABLE 1. *Second-order degradation rate constants for three compounds in water*

Compound	k (l organism^{-1} h^{-1})[a]	N[b]	Temp (°C)[c]
2,4–D[d] butoxyethyl ester	$(5.4 \pm 0.50) \times 10^{-10}$	31	18.8 ± 6.6 (1–29)
Malathion	$(4.4 \pm 0.66) \times 10^{-11}$	14	21.1 ± 7.8 (2–27)
Chlorpropham	$(2.4 \pm 0.42) \times 10^{-14}$	11	19.1 ± 5.6 (7–28)

[a] Mean across all sites and all concentrations ± relative standard deviation.
[b] Number of sites for which k was determined.
[c] Average temperature ± standard deviation. Value in parentheses is the range of temperature.
[d] 2,4–D, 2,4–Dichlorophenoxyacetic acid.

of the mean and standard deviation (Table 1) across all sites clearly shows that the mean value is highly independent of site. Studies at different concentrations of substrate and organisms confirmed that the rate is first order both in substrate and in population size for all cases examined. This surprisingly high level of predictability raises many questions which can only be answered by many more data. It does offer significant encouragement, however, for prediction of microbial transformation kinetics.

TABLE 2. *Rate constants for transformation in sediment-water systems*

Compound	Fraction sorbed	k_{aq} (l organism^{-1} h^{-1})	k_2 (l organism^{-1} h^{-1})
2,4-D butoxyethyl ester[a]	0[b]	$(2.3 \pm 0.8) \times 10^{-10}$	
Malathion[c]	0[b]	$(4.0 \pm 0.9) \times 10^{-11}$	
Chlorpropham[c]	0[b]	$(1.42 \pm 0.6) \times 10^{-14}$	
	0.15	$(4.15 \pm 0.7) \times 10^{-14}$	$(3.45 \pm 0.2) \times 10^{-15}$
	0.90	$(7.4 \pm 1.2) \times 10^{-15}$	$(2.29 \pm 0.6) \times 10^{-15}$
Methoxychlor[c]	0[b]	$(6.7 \pm 1.2) \times 10^{-14}$	
	0.15	$(5.2 \pm 2.3) \times 10^{-14}$	$(3.0 \pm 0.5) \times 10^{-14}$
	0.90	$(6.1 \pm 2.8) \times 10^{-15}$	$(3.2 \pm 1.2) \times 10^{-14}$
Bis(2-ethylhexyl) phthalate[c]	0[b]	$(4.2 \pm 0.7) \times 10^{-13}$	
	0.15	$(1.8 \pm 0.3) \times 10^{-13}$	$(3.6 \pm 1.3) \times 10^{-13}$
	0.90	$(5.7 \pm 1.7) \times 10^{-17}$	$(6.1 \pm 2.2) \times 10^{-13}$
Dibutyl phthalate[c]	0[b]	$(3.1 \pm 0.8) \times 10^{-11}$	
	0.15	$(2.7 \pm 1.5) \times 10^{-11}$	$(3.5 \pm 1.7) \times 10^{-11}$
	0.90	$(6.1 \pm 0.5) \times 10^{-14}$	$(2.6 \pm 0.2) \times 10^{-11}$
Di(*n*-octyl) phthalate[c]	0[b]	$(3.7 \pm 0.6) \times 10^{-11}$	
	0.15	$(2.1 \pm 1.2) \times 10^{-11}$	$(3.6 \pm 0.7) \times 10^{-11}$
	0.90	$(3.1 \pm 0.4) \times 10^{-15}$	$(6.9 \pm 2.3) \times 10^{-11}$

[a] Data from W. C. Steen (in preparation). 2,4-D, 2,4-Dichlorophenoxyacetic acid.
[b] Sediment free.
[c] Data from Steen et al. (13).

These measurements were made by following the disappearance of the parent compound, which in effect defines transformation. Gilbert and Watson (7) call this primary or functional biodegradation, i.e., biodegradation of a substance to an extent sufficient to remove some characteristic property of the molecule. It should be noted that the rate of primary transformation will probably not be equal to the rate of mineralization or loss of absorbance, etc. For preliminary assessment of environmental behavior, the rate of primary degradation will usually be the parameter of choice. For other purposes such as treatability, mineralization rate may well be required.

The question of how the active population, [*B*], should be measured is controversial. Jannasch and Jones (8), Kangas and Coleman (9), and others have compared different methods. It is unlikely that this question has a single best answer. The only real requirement is that the method be adequately reproducible and comparable from one site or study to another. The data in Table 1 indicate that, at least for these compounds, plate counts are adequate.

For degradation studies in suspended, aerobic, sediment systems, plate counts cannot be used, and it was found to be necessary to resort to adenosine triphosphate measurement (13). Table 2 shows the rate constants obtained via equation 5:

$$-\frac{d[S]_T}{dt} = \frac{k}{1 + \rho K_p}[B][S]_T = k_{app}[B][S]_T \qquad (5)$$

where k is the second-order rate constant from equation 4, ρ is the sediment-

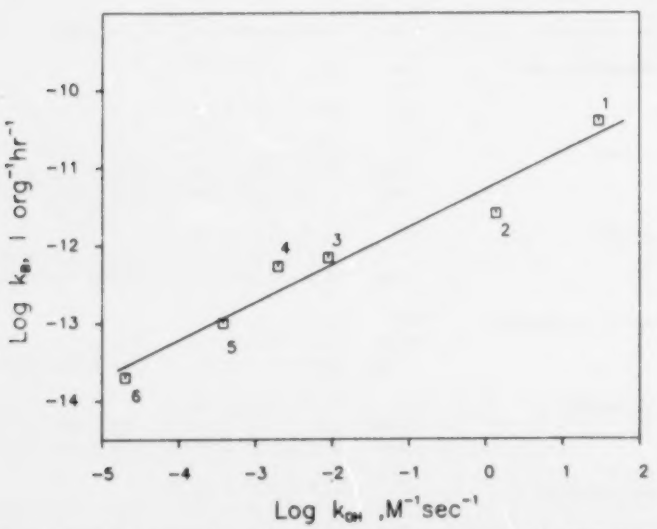

FIG. 1. *Correlation of second-order alkaline hydrolysis rate constants (27°C) determined in distilled water using second-order biolysis rate constants determined in natural water samples. The compounds are (1) n-butoxylethyl ester of 2,4-D (2,4-dichlorophenoxyacetic acid), (2) malathion, (3) methyl benzoate, (4) methyl anisate, (5) methoxychlor, (6) chlorpropham.*

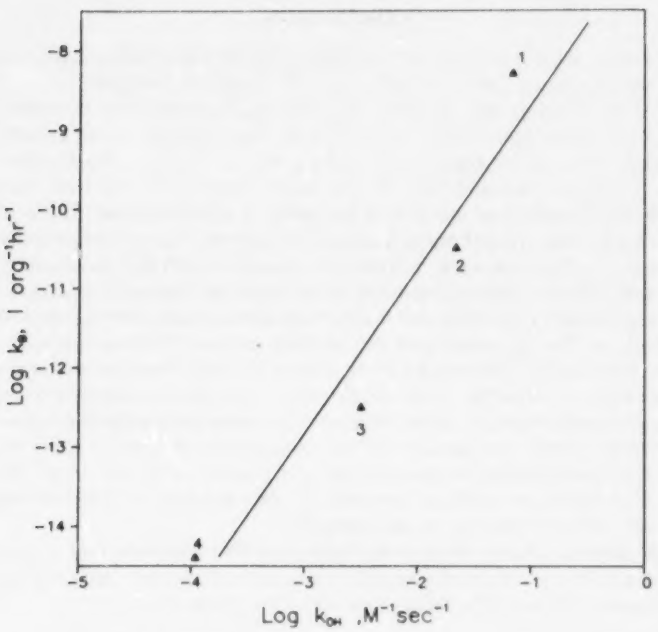

FIG. 2. *Correlation of second-order alkaline hydrolysis rate constants (30°C) obtained in distilled water with second-order biolysis rate constants (25°C). (1) Dimethyl phthalate, (2) di(n-butyl) phthalate, (3) di(n-octyl) phthalate; (4) di(2-ethylhexyl) phthalate.*

to-water mass ratio, K_p is the partition coefficient of substrate between sediment and water, k_{app} is the apparent second-order rate constant, and $[S]_T$ is the total amount of substrate per unit volume of the system.

These data indicate that, when population size and partitioning are included, the process is again first order in $[B]$ and $[S]$. Also, the data for the compounds in Table 1 show the second-order rate constants to be about the same whether measured with water column organisms or with sediment populations in the absence of sediment. Further, the influence of sediments on degradation of highly insoluble (hydrophobic) compounds is clearly expected to be a lowered rate, all other things being equal.

If rate constants that are system independent can be measured, then it may be possible to develop relationships between the rate constants and properties that reflect molecular structure. The use of linear free energy (structure-activity) relationships has been well known for many years in chemistry and more recently in biology. Figures 1 and 2 show the relationships between the second-order biolysis rate constant and the abiotic second-order alkaline hydrolysis rate constant (14). The good correlation of these data over so many orders of magnitude lends considerable credibility and promise to the potential utility of this approach. Why such a good correlation exists is not clear at this time, but the explanation may well be because the microbial transformation is hydrolytic for these compounds.

CONCLUSION

Application of kinetics to the problem of pollutant transformation in the environment requires that we formulate the equations in terms of substrate disappearance rather than growth. Also, the problem must be considered in the context of multiple carbon sources; i.e., the substrate is not growth limiting. Data have been presented to show that a simple second-order rate equation can be used and that, for the study compounds, the rate constant is highly independent of the system for which it was measured. These observations leave unanswered many questions about the role of temperature, cometabolism, other nutrients, population structure, and the structure of the compound. There is little compelling evidence in the literature to suggest that a similar approach cannot be taken with most compounds. On the other hand, confidence in the approach and the ultimate answer to these questions can only be obtained by additional kinetic studies for many compounds and sites. Perhaps most importantly, future studies must incorporate statistical treatment of data and mathematical analysis according to some proposed rate equations. Unless this is done, no basis exists for comparison of results. After all, the most important question for our purpose is not whether the rate be predicted; rather, it is necessary to know how well the rate can be predicted in order to determine whether the results are adequate.

Finally, kinetic models as outlined here provide a rationale that is essential for development of methodologies to rigorously assess the role of microbial transformation in both the laboratory and the environment.

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